residues. The RACE product was subsequently cloned. All three clones containing SM38 sequence (Two PCR generated clones and one clone from the S.mansoni cDNA library) were contiguous and overlapping. When assembled, the SM38 sequence included 1049 bp of sequence including 5' untranslated sequence, two potential initiation methionines, an open reading frame encoding a 303 amino acid protein, a stop codon, 3' untranslated sequence and a poly-adenylation site.

## REMARKS

Applicants submit herewith an initial Sequence Listing in computer and paper form in accordance with 37 C.F.R. §1.821-1.825. The content of the paper and computer readable copies of the Sequence Listing submitted in accordance with 37 C.F.R. §1.821(c) and (e) are the same and do not include new matter. Also enclosed is a copy of the Notice to Comply.

In accordance with the Notice to Comply, the sequences depicted in Figures 13-17 have been included in the sequence listing.

The specification has been amended to include sequence identifiers in the preceding "IN THE SPECIFICATION" section. Attached hereto is a marked-up version of the changes made to the specification paragraphs. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE" and is only included for the Examiner's convenience. Should any discrepancies be discovered, the version presented in the preceding "IN THE SPECIFICATION" section shall take precedence.

## CONCLUSION

Please charge any fees associated with this filing or credit any overpayment to Deposit Account No. 02-4377. A duplicate copy of this paper is enclosed.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION

Paragraph 26 on pp.12-13 has been **amended** as follows:

Figure 13. Comparison of *S. mansoni* SM38 cDNA with *S. mansoni* ESTs. The SM38 cDNA (SEQ ID NO:7) was isolated and cloned from an *S. mansoni* cDNA library as described in methods. The cDNA for SM38 includes 5' untranslated sequence (gray box), an initiation methionine (underlined), an open reading frame encoding a 303 amino acid protein (clear box), a stop codon (underlined), 3' untranslated sequence (gray box) and a poly-adenylation site (underlined). The full-length cDNA was compared to published *S. mansoni* EST sequences and three separate EST sequences (SEQ ID NOS:4-6) were found that were identical to portions of the SM38 sequence. The SM38 cDNA includes 421 base pairs of unique sequence (70 bp 5' untranslated and 351 bp of open reading frame) not found in any public database.

Paragraph 27 on p.13 has been **amended** as follows:

Figure 14. Translation of SM38 cDNA. The SM38 cDNA (SEQ ID NO:1) was translated in all reading frames and an open reading frame of 303 amino acids (SEQ ID NO:2) was identified. The initiation codon is located at nucleotide position 71-73 and the termination codon is found at nucleotide position 980-983.

Paragraph 28 on pp.13-14 has been **amended** as follows:

Figure 15. SM38 is homologous to Aplysia ADP-ribosyl cyclase and human CD38 cyclase. The protein sequence of SM38 (SEQ ID NO:10) was aligned with the protein

sequences for Aplysia ADP-ribosyl cyclase (part a) (SEQ ID NO:8) and human ADP-ribosyl cyclase CD38 (part b) (SEQ ID NO:9). A high degree of homology (boxed residues) was observed with 21% identity between the Aplysia protein and SM38 and 23% identity between human CD38 and SM38. The conserved 10 cysteine residues present in all members of the cyclase protein family are also present in SM38 (shaded boxes). The two additional cysteines found in CD38 (underlined), but not in Aplysia, are also lacking in SM38. However, the SM38 protein contains two additional cysteine residues that are unique and are not found in either CD38 or Aplysia cyclase (underline). Most importantly, the active site catalytic residues identified for CD38 and Aplysia enzyme (starred residues) are also present in SM38.

Paragraph 29 on p.14 has been **amended** as follows:

Figure 16. SM38 is a soluble protein. The protein sequence of SM38 (SEQ ID NO:10) was examined to determine if the protein is a type-II membrane bound protein like CD38, a soluble protein like the Aplysia cyclase, a GPI-linked protein like other cyclase family proteins, or a secreted protein. The conserved enzyme domain (see previous figure) is shaded. SM38 contains only 22 amino acids 5' of the enzyme domain. These 22 amino acids are not hydrophobic, thus, no leader sequence 5' of the enzyme domain could be identified, indicating that SM38 is not secreted or GPI-linked. Additionally, no 5' transmembrane domain could be identified, indicating that SM38 is not a type-II membrane protein. Therefore, SM38 is most likely a soluble cytoplasmic protein like Aplysia cyclase.

Paragraph 30 on p.14 has been **amended** as follows:

Figure 17. Reverse translation of SM38. The 303 amino acid coding region of SM38 was reverse-translated to identify a degenerate DNA sequence that would encode the SM38 protein (SEQ ID NO:11).

Paragraph 35 on p.16 has been **amended** as follows:

The cDNA sequence (SEQ ID NO:1) and deduced amino acid sequence (SEQ ID NO:2) of *S. mansoni* SM38 is shown in Figure 14 (ATCC Deposit No: ). The SM38 cDNA was translated in all reading frames and an open reading frame of 303 amino acids was identified. The initiation codon is located at nucleotide position 71 and the termination codon is found at nucleotide position 981.

Paragraph 151 on pp.73-75 has been **amended** as follows:

Primers were made corresponding to the EST sequence found in Genbank accession #AW017229. (5' primer: acatctttgtggtactgaatggctcgg and 3' primer: tgagtaatgtctcgacgtttgacctcg) (SEQ ID NOS:12-13). *S. mansoni* cDNA libraries were obtained from Dr. Phillip LoVerde (SUNY, Buffalo), and were subjected to PCR using the primers indicated above. The library (1-20 μl) and dH2O were heated to $70^0$C for 10 minutes and were then combined with the remainder of the PCR reagents and cycled. The cycles were: $95^0$C 5 minutes, 1 cycle, followed by $95^0$C 1 minute, $65^0$C 1 minute and $72^0$C 2 minutes for 35 cycles followed by 1 cycle at $72^0$C for 5 minutes. The expected 330 bp band corresponding to EST AW017229 was isolated, TOPO cloned, and then used as a probe to screen 250,000 plaques from the *S. mansoni* cDNA library. Five positives were isolated and then subjected to 3 more rounds

of screening in order to produce plaque pure clones. All five clones were fully sequenced on both strands. The nucleotide sequence and amino acid translation of four of the clones were identical (referred to as SM38). The stop codon and polyadenylation sites were identified in all of the SM38 clones, but the initiation methionine was not present in any of the clones. To obtain the 5' end of the SM38 gene, a single primer extension approach (NAR, 1994, vol 22, No. 16, p3427-3428) was utilized. A first round of PCR was performed using an external SM38 primer (5' catcgaataaccctgatttcataacac) (SEQ ID NO:14) and the universal reverse primer for Bluescript. Two μl of this reaction was then subjected to PCR using an internal nested SM38 primer (5' gataaagtaagaactcgtgcc) (SEQ ID NO:15) and the universal reverse primer. A 200 and a 300 bp band were identified from this reaction and were directly sequenced. The sequence obtained overlapped 124 bp with the 5' end of the SM38 clones and included an additional 153 bp of sequence, however the no stop codon was detected, indicating that we still did not have the 5' end of the gene. Therefore, classic 5"RACE (PNAS vol 85 pp 8998-9002, Dec. 1998) was performed using using cDNA prepared from RNA isolated from adult *S. mansoni* worms (RNA provided by Dr. P. LoVerde, SUNY Buffalo). 10X Taq buffer, dNTP's, cDNA and Expand High Fidelity Taq were combined with the dT-AP primer (see ref. For details) and cycled for 5 minutes at 95°C followed by 2 minutes at 50°C and 40 minutes at 72°C. After this 40 minute incubation the 5' external SM38 primer (see above) and AP primers were added and cycled for 35 cycles under the conditions: 95°C for 15 sec, 47°C for 30 sec, 72°C for 2 minutes followed by a 5 minute extension at 72°C. The reactions were run on a 1.5% agarose gel and a 300 bp band was isolated using Qiagen Gel Kit. The 5' RACE product was directly sequenced with the AP and 5' external SM38 primer. Two potential initiation methionines were identified in the

sequence and two stop codons were found 13-19 amino acids upstream of the methionine residues. The RACE product was subsequently cloned. All three clones containing SM38 sequence (Two PCR generated clones and one clone from the S.mansoni cDNA library) were contiguous and overlapping. When assembled, the SM38 sequence included 1049 bp of sequence including 5' untranslated sequence, two potential initiation methionines, an open reading frame encoding a 303 amino acid protein, a stop codon, 3' untranslated sequence and a poly-adenylation site.

## CONCLUSION

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